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ORIGINAL ARTICLE

HCRP1 expression status is a significant prognostic marker in oral and oropharyngeal cancer

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OBJECTIVE: The hepatocellular carcinoma-related protein 1 (HCRP1) is a key factor in the degradation of the epidermal growth factor receptor. In this study, we assessed the prognostic significance of HCRP1 expression in patients with oral and oropharyngeal squamous cell carcinoma (OOSCC).

METHODS: HCRP1 expression was determined by immunohistochemistry on tissue biopsy sections of 111 patients with locally advanced OOSCC undergoing neoadjuvant chemoradiotherapy followed by surgery. The Kaplan–Meier method and Cox regression models were used for survival analyses.

RESULTS: Low HCRP1 expression was associated with poor recurrence-free survival ($P = 0.046$) and overall survival ($P = 0.03$). Multivariate analysis revealed that low HCRP1 expression remained an independent risk factor for relapse (HR 2.98, 95% CI 1.19–7.49, $P = 0.02$) and death (HR 3.04, 95% CI 1.19–7.79, $P = 0.02$).

CONCLUSION: Low HCRP1 expression was found to be of adverse prognostic significance in patients with OOSCC who received preoperative chemoradiotherapy.

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Keywords: HCRP1; epidermal growth factor receptor; immunohistochemistry; oral and oropharyngeal cancer; prognosis

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cancer worldwide and is responsible for approximately 350 000 deaths annually (Ferlay *et al*, 2010). Despite improvements in the treatment of HNSCC in the last decades, the overall survival of these patients remains poor (Farshadpour *et al*, 2007). Molecular-targeted therapies and predictive biomarkers to select those patients who benefit promise to further improve outcome (Scully and Bagan, 2009b).

Deregulation of epidermal growth factor receptor (EGFR) signalling is a frequent event in HNSCC (Kalyankrishna and Grandis, 2006; Diniz-Freitas *et al*, 2007), and the inhibition of EGFR, for example by monoclonal antibodies such as cetuximab, is an important treatment strategy (Vermorken *et al*, 2008; Scully and Bagan, 2009a; Vermorken and Specenier, 2010). Although there are several mechanisms to modulate EGFR signalling, the EGFR downregulation process plays a key role in controlling receptor overexpression and downstream transduction of deregulated signals (Karamouzis *et al*, 2007). This downregulation process is determined by EGFR sorting into the intraluminal vesicles of multivesicular bodies (MVBs), which leads to MVBs fusing with lysosomes and finally to the degradation of EGFR by lysosomal enzymes (Katzmann *et al*, 2002). The human endosomal sorting complex required for transport-1 consists of the four subunits, Mvb12 (multivesicular body sorting factor 12), Tsg101 (tumour susceptibility gene 101), hVps28 (vacuolar protein sorting 28 homologue) and HCRP1 [hepatocellular carcinoma-related protein 1, also known as vacuolar protein sorting 37 homologue A (hVps37A)], and is responsible for the binding and sorting of ubiquitinated receptors into intraluminal vesicles of MVBs and the subsequent degradation in lysosomes (Bache *et al*, 2004; Kostelansky *et al*, 2007).

Recently, it has been shown that HCRP1 is downregulated in a large number of ovarian cancers and that loss of expression is associated with cetuximab resistance in vitro (Wittinger *et al*, 2011). Because EGFR is frequently overexpressed and is also associated with poor survival in HNSCC (Rubin Grandis *et al*, 1998; Cohen, 2006), downregulation of HCRP1 may also play an important role in the pathogenesis of this malignancy.

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The purpose of this study was to evaluate the clinical relevance of HCRP1 expression in patients with oral and oropharyngeal squamous cell carcinoma (OOSCC) who received preoperative chemoradiotherapy.

Materials and methods

Study population and treatment

Medical charts of patients with primary locally advanced oral and oropharyngeal cancer who were treated with neoadjuvant chemoradiation followed by cancer surgery at the Departments of Radiotherapy and Cranio-Maxillofacial and Oral Surgery, at the Medical University of Vienna, between 2000 and 2009, were retrospectively reviewed. Patients suitable for inclusion in this study had to meet the following criteria: (i) biopsy-confirmed primary OOSCC, (ii) no previous treatment for OOSCC, (iii) disease Tumour node metastasis (TNM) stages III and IV, (iv) no distant metastatic disease (M0), (v) no previous history of HNSCC, (vi) World Health Organization (WHO) performance status and laboratory parameters allowing chemotherapy and surgery, (vii) curative-intent treatment with neoadjuvant chemoradiotherapy followed by loco-regional tumour surgery, and (viii) clear resection margins (R0). A total of 176 patients fulfilled the aforementioned inclusion criteria. Of those, 65 patients were excluded because pretreatment biopsy tumour blocks were not available, leaving a total of 111 patients for analysis. Excluded and included patients were similar with respect to demographic characteristics and survival rates (data not shown). The multimodal therapy was decided on in a multidisciplinary team meeting following the institutional protocol as described previously (Perisanidis *et al*, 2012). Briefly, it consists of a neoadjuvant chemotherapy regimen of mitomycin C (15–20 mg m^{-2}, i.v. bolus injection on day 1), 5-fluorouracil (750 mg m^{-2} per day, continuous infusion on days 1–5) and concurrent radiation administered up to a total dose of 50 Gy (25 fractions of 2 Gy per day). Surgery was performed 4–8 weeks after the completion of radiation therapy. Follow-up was performed over a period of 5 years after surgery (in 3-month intervals during the first 2 years and then in 6-month intervals for the next 3 years).

A patient database was generated using demographic and clinical data extracted from the Vienna General Hospital Patient Information System (AKIM) together with data obtained from surgical and pathologic reports. Tumour node metastasis staging was based on the International Union Against Cancer Classification. The WHO classification was used to determine the histologic tumour type and grading. Pathologic tumour response was evaluated as described (Braun *et al*, 1989), according to vitality of tumour cells in surgical specimens: no vital tumour cells, <5% of vital tumour cells, 5–50% of vital tumour cells and more than 50% of vital tumour cells [regression grades (RG) 1, 2, 3 and 4, respectively]. Grades were grouped as responder (RG1/RG2) and non-responder (RG3/RG4). The study was approved by the local Institutional Review Board.

Immunohistochemistry and evaluation of staining

Immunohistochemistry (IHC) was performed in an ISO certified laboratory of the Department of Medicine I, Medical University of Vienna. All tumour specimens were obtained at the time of surgery before neoadjuvant therapy. Tumour blocks were archived at the Department of Pathology at the Medical University of Vienna. Participating pathologists, blinded to patient clinical outcome, were asked to provide all formalin-fixed paraffin-embedded biopsy tissue blocks and their corresponding haematoxylin and eosin (H&E)-stained sections. Corresponding H&E sections of all blocks were reviewed to choose a single, representative tissue block with the greatest amount/area of carcinoma.

HCRP1 protein expression was immunohistochemically analysed by means of a standard protocol. In brief, 4-μm tissue sections were cut and deparaffinized with xylene, rehydrated in graded ethanol and incubated for 10 min with 0.3% H_2O_2. Antigen retrieval was performed by boiling sections in a pressure cooker for 10 min in 10 mM citrate buffer (pH 6.0). To reduce background staining, sections were incubated with Ultra V block [UltraVision LP detection system; Lab Vision Corporation (Thermo Fisher Scientific Inc., Fremont, CA, USA)]. After incubation with the HCRP1-specific monoclonal antibody for 60 minutes at room temperature (clone EGT290, dilution 1:2500, Eurogentec SA, Seraing, Belgium), antibody binding was detected using the UltraVision LP detection system according to the manufacturer's recommendations (Lab Vision Corporation). Finally, colour development was performed by 3,3 diaminobenzidine and counterstaining by haematoxylin.

An experienced pathologist (T. W.), blinded to patient characteristics and outcome, evaluated both staining intensity and frequency of immunostaining for each tumour using light microscopy. For each patient, one biopsy tumour slide was reviewed. Staining intensity was scored in four categories: no staining (0), weak (1 +), intermediate (2 + , between 1 + and 3 +) and strong (3 +). The study cohort was dichotomized into low or high HCRP1 expression groups. High HCRP1 expression was defined as >10% tumour cells with at least 2 + or + 3 intensity as previously described (Wittinger *et al*, 2011).

Statistical analysis

The primary endpoint of this analysis was overall survival (OS) defined as the time from surgery to death from any cause. Recurrence-free survival defined as the time between surgery and cancer recurrence (loco-regional or distant recurrence) or death without recurrence was a secondary endpoint.

Baseline data according to dichotomized HCRP1 status were compared with univariate analyses using the Mann–Whitney *U* test for continuous variables and the chi-square test for categorical variables. Survival rates were estimated by means of the Kaplan–Meier method, and the log-rank test was used to analyse survival differences between groups. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using multivariate Cox proportional hazards regression models to analyse the independent impact of clinicopathologic factors and HCRP1 on

survival. A *P*-value equal to or <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS®, version 19.0; SPSS Inc., Chicago, IL, USA).

Results

HCRP1 expression and patient characteristics

The characteristics of the study cohort are shown in Table 1. For the HCRP1 immunohistochemical evaluation, sufficient tissue was available from all 111 patients. High expression of HCRP1 was observed in 100 of 111 tumours (90%). Representative examples of HCRP1 immunostaining with control slides are shown in Figure 1 (Wittinger *et al*, 2011). HCRP1 expression was not significantly associated with clinicopathologic factors such as age, sex, smoking, alcohol consumption, tumour localization, tumour grade, ypT, ypN and perineural invasion (Table 1).

Table 1 Associations between HCRP1 expression status and clinicopathologic variables of 111 patients with oral and oropharyngeal cancer

Variable	*All patients No. (%)*	*Low HCRP1 No. (%)*	*High HCRP1 No. (%)*	P-*value*[a]
All	111 (100)	11 (10)	100 (90)	
Age				
Median, years	58	56	58	0.80[b]
Range, years	24–79	37–71	24–79	
Sex				
Male	82 (74)	7 (64)	75 (75)	0.47
Female	29 (26)	4 (36)	25 (25)	
Smoking				
Current	92 (83)	9 (82)	83 (83)	0.92
Former or never	19 (17)	2 (18)	17 (17)	
Alcohol consumption				
Current	81 (73)	10 (91)	71 (71)	0.28
Former or never	30 (27)	1 (9)	29 (29)	
Tumour localization				
Oral cavity	95 (86)	10 (91)	85 (85)	0.60
Oropharynx	16 (14)	1 (9)	15 (15)	
Tumour grade				
G1	10 (9)	2 (18)	8 (8)	0.28
G2	88 (79)	9 (82)	79 (79)	
G3	13 (12)	0 (0)	13 (13)	
ypT				
ypT0	56 (50)	7 (64)	49 (49)	0.41
ypT1	32 (29)	2 (18)	30 (30)	
ypT2	11 (10)	0 (0)	11 (11)	
ypT3	3 (3)	1 (9)	2 (2)	
ypT4	9 (8)	1 (9)	8 (8)	
ypN				
ypN0	83 (75)	8 (73)	75 (75)	0.67
ypN1	23 (21)	3 (27)	20 (20)	
ypN2	6 (5)	0 (0)	5 (5)	
Perineural invasion				
Negative	99 (89)	9 (82)	90 (90)	0.34
Positive	12 (11)	2 (18)	10 (10)	
Pathologic response				
RG1	56 (50)	7 (64)	49 (49)	0.75
RG2	15 (14)	1 (9)	14 (14)	
RG3	5 (4)	0 (0)	5 (5)	
RG4	35 (32)	3 (27)	32 (32)	

ypT, pathologic T after neoadjuvant therapy; ypN, pathologic N after neoadjuvant therapy; RG, regression grade.
Percentages may not total 100 because of rounding.
aChi-square test unless otherwise specified.
bMann–Whitney U test.

Correlation of HCRP1 expression with pathologic tumour response

All 111 patients were treated with neoadjuvant chemoradiotherapy followed by loco-regional tumour surgery, and all were evaluable for pathologic response. The response rate (RG1/RG2) of the study population was 64%. In the responder group, 56 (50%) patients were categorized as RG1 and 15 (14%) as RG2. In the non-responder group, 5 (4%) patients were found to be RG3 and 35 (32%) RG4. No statistically significant association between HCRP1 expression and pathologic response to neoadjuvant chemoradiotherapy was observed. The response rate was 73% for patients with low HCRP1 expression and 63% for those with high HCRP1 expression ($P = 0.75$) (Table 1).

HCRP1 and survival

The median follow-up of the total study population was 4.8 years, and the maximum follow-up was 10.4 years. Fifty-five patients died (seven patients with low HCRP1 expression and 48 patients with high HCRP1 expression). The estimated 2-year and 5-year overall survival rates of all 111 patients were 66% and 46%, respectively. The recurrence-free survival rate was 62% at 2 years and 45% at 5 years.

In univariate survival analyses, age (log-rank $P = 0.03$), ypT (log-rank $P < 0.001$), ypN (log-rank $P = 0.005$), perineural invasion (log-rank $P = 0.005$) and pathologic tumour response (log-rank $P < 0.001$) were significantly associated with recurrence-free survival. Age (log-rank $P = 0.007$), alcohol consumption (log-rank $P = 0.041$), ypT (log-rank $P < 0.001$), ypN (log-rank $P = 0.009$), perineural invasion (log-rank $P = 0.006$) and pathologic tumour response (log-rank $P = 0.001$) were significantly associated with overall survival (Table 2).

Kaplan–Meier analysis revealed that patients with low HCRP1 expression had a significantly shorter recurrence-free survival compared to patients with high HCRP1 expression (log-rank $P = 0.046$) (Figure 2). The recurrence-free survival probability at 5 years was 34% for patients in the low HCRP1 expression group and 46% for patients in the high HCRP1 expression group. Furthermore, patients with low HCRP1 expression had a significantly shorter overall survival than those with high HCRP1 expression (log-rank $P = 0.03$) (Figure 3). The overall survival probability at 5 years was 28% for patients in the low HCRP1 expression group and 48% for patients in the high HCRP1 expression group.

The independent impact of HCRP1 expression on recurrence-free survival and overall survival was assessed by Cox regression models adjusted for age, sex, smoking, alcohol consumption, tumour localization, tumour grade, ypT, ypN, perineural invasion and pathologic response (Table 2). In these analyses, low HCRP1 expression remained significantly associated with shorter recurrence-free survival (HR 2.98, 95% CI 1.19–7.49, $P = 0.02$) and shorter overall survival (HR 3.04, 95% CI 1.19–7.79, $P = 0.02$). Thus, low HCRP1 expression is an independent poor prognostic factor in patients with OOSCC who received preoperative chemoradiotherapy.

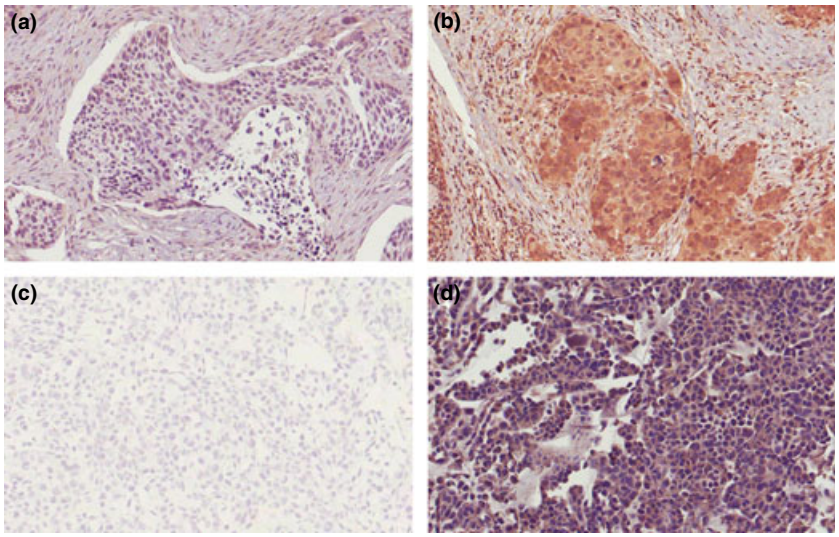

Figure 1 Representative examples of HCRP1 immunohistochemical staining. (**a**) low HCRP1; (**b**) high HCRP1; (**c**) negative control (MDAH-2774 ovarian cancer cell line, HCRP1 mRNA is silenced by stable HCRP1-specific shRNA expression); (**d**) positive control (MDAH-2774 ovarian cancer cell line, endogenous expression of HCRP1). Magnification 40 ×

Table 2 Univariate and multivariate survival analyses in 111 patients with oral and oropharyngeal cancer

	Recurrence-free survival			*Overall survival*		
	Univariate[b]	*Multivariate*[c]		*Univariate*[b]	*Multivariate*[c]	
Variable[a]	P-*value*	P-*value*	*HR (95% CI)*	P-*value*	P-*value*	*HR (95% CI)*
HCRP1	0.046	0.02	2.98 (1.19–7.49)	0.03	0.02	3.04 (1.19–7.79)
Age (years)	0.03	0.04	1.03 (1.00–1.06)	0.007	0.02	1.04 (1.01–1.07)
Sex	0.20	0.39	1.34 (0.68–2.62)	0.22	0.58	1.22 (0.62–2.40)
Smoking	0.29	0.33	1.50 (0.66–3.41)	0.38	0.63	1.22 (0.55–2.72)
Alcohol consumption	0.08	0.11	1.79 (0.87–3.66)	0.041	0.07	1.96 (0.94–4.08)
Tumour localization	0.08	0.39	1.49 (0.60–3.68)	0.06	0.30	1.62 (0.66–3.99)
Tumour grade	0.45	0.57	0.84 (0.45–1.55)	0.31	0.40	0.76 (0.40–1.44)
ypT	<0.001	0.01	1.53 (1.10–2.12)	<0.001	0.06	1.36 (0.99–1.87)
ypN	0.005	0.006	1.85 (1.20–2.86)	0.009	0.004	1.96 (1.24–3.11)
Perineural invasion	0.005	0.89	1.07 (0.45–2.55)	0.006	0.95	1.03 (0.42–2.51)
Pathologic response	<0.001	0.60	1.10 (0.77–1.55)	0.001	0.49	1.13 (0.80–1.60)

Tumour grade, ypT, ypN and pathologic response are ordinal variables.
HR, hazard ratio; CI, confidence interval; ypT, pathologic T after neoadjuvant therapy; ypN, pathologic N after neoadjuvant therapy.
[a]Variables were analysed as follows: HCRP1, low vs high (reference); age (years), continuous variable; sex, male vs female (reference); smoking, current vs former or never (reference); alcohol consumption, current vs former or never (reference); tumour localization, oral cavity vs oropharynx (reference); tumour grade, G1 vs G2 vs G3; ypT, ypT0 vs ypT1 vs ypT2 vs ypT3 vs ypT4; ypN, ypN0 vs ypN1 vs ypN2; perineural invasion, positive vs negative (reference); pathologic response; RG1 vs RG2 vs RG3 vs RG4.
[b]Log-rank test.
[c]Multivariate Cox regression analysis; HRs adjusted for all listed variables.

Discussion

In our work, we examine the prognostic significance of HCRP1 expression status in a cohort of patients with locally advanced oral and oropharyngeal cancer. The results of this study provide evidence that downregulation of HCRP1 expression is associated with a shorter survival in patients with OOSCC undergoing preoperative chemoradiotherapy.

The prognostic value of HCRP1 has already been demonstrated in primary liver and ovarian cancer. In hepatocellular carcinoma, low HCRP1 mRNA expression was independently associated with shorter disease-free survival (Lai *et al*, 2009). In a recent study of ovarian cancer, it was reported that HCRP1 expression had a significant impact on the prognostic value of EGFR expression (Wittinger *et al*, 2011). High EGFR expression in particular was significantly associated with decreased overall survival only in tumours with low or missing HCRP1 expression. In contrast, no significant correlation between high EGFR expression and all-cause mortality was found in tumours with high HCRP1 expression. In accordance with these studies, we revealed that OOSCC patients with low HCRP1 expression had a significantly shorter overall and recurrence-free survival compared to patients with high HCRP1 expression. A multivariate analysis confirmed that low HCRP1 expression remained an independent risk factor for death ($P = 0.02$) and disease recurrence ($P = 0.02$).

Epidermal growth factor receptor is expressed in the majority of HNSCCs leading to the downstream activation of several signalling pathways that play a pivotal role in

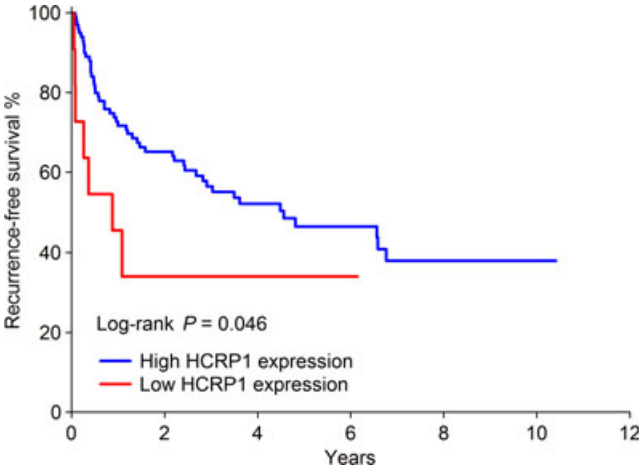

Figure 2 Kaplan–Meier estimates of the probability of recurrence-free survival according to low and high HCRP1 expression of 111 patients with locally advanced oral and oropharyngeal squamous cell carcinoma

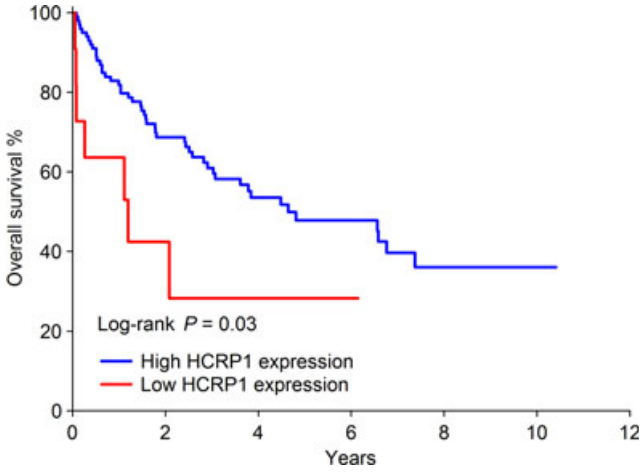

Figure 3 Kaplan–Meier estimates of the probability of overall survival according to low and high HCRP1 expression of 111 patients with locally advanced oral and oropharyngeal squamous cell carcinoma

regulating cell proliferation, inhibition of apoptosis, angiogenesis, invasion and metastasis (Ciardiello and Tortora, 2003; Silva *et al*, 2010). Furthermore, high expression of EGFR has been shown to correlate with reduced survival in patients with HNSCC (Rubin Grandis *et al*, 1998). Thus, EGFR has emerged as an attractive molecular target in HNSCC therapy. To date, EGFR signalling inhibition strategies are widely used in HNSCC clinical trials (Cohen, 2006). However, results of clinical trials suggest that after EGFR inhibition, only a small subset of patients (4–26%) achieve objective response (Cohen *et al*, 2003; Soulieres *et al*, 2004; Egloff and Grandis, 2008). Cumulative evidence suggests that most patients who initially respond to EGFR inhibitors will eventually develop acquired therapeutic resistance (Pao *et al*, 2005; Wheeler *et al*, 2008; Chen *et al*, 2010). The potential mechanisms of intrinsic or acquired resistance to EGFR-targeted therapies involve EGFR mutations (EGFRvIII), mutations in downstream signalling components (PI3K and PTEN mutations) and concurrent activation of other receptor tyrosine kinases that maintain the activation of EGFR downstream pathways (Barnes *et al*, 2007; Karamouzis *et al*, 2009; Chen *et al*, 2010). In addition, Wheeler and colleagues (Wheeler *et al*, 2008) have shown that cetuximab-resistant cancer cells, derived from either HNSCC or NSCLC lines, have an impaired ability to internalize and degrade the EGFR, which results in an increased steady-state expression of EGFR. This increase in EGFR expression leads eventually to hyperactivation of HER3 and subsequent activation of the PI3K/AKT pathway. Recently, the role of HCRP1 status in the development of resistance after EGFR inhibition with cetuximab has been highlighted (Wittinger *et al*, 2011). It was reported that in HCRP1-negative ovarian cancer cells, acquired resistance to cetuximab is linked to the accumulation of activated EGFR in the cytoplasm because of HCRP1-related defects in receptor degradation. The endosomally located phosphorylated EGFR retains its capability to activate signalling cascades, and this results in the activation of downstream pathways, as evidenced by increased phosphorylation of ERK1/2 and sustained baseline AKT phosphorylation. In contrast, EGFR inhibition by lapatinib resulted in ERK1/2 and AKT dephosphorylation independent of HCRP1 status. In this context, further studies are required to investigate the possible link between HCRP1 expression and acquired resistance to cetuximab therapy also in patients with OOSCC.

In our investigation, we used strict eligibility criteria to construct a cohort of homogeneous and uniformly treated OOSCC patients. Nevertheless, this study has clear limitations. First, the survival analysis was based on a relatively small sample size. Second, surgery followed by radiotherapy or primary concomitant chemoradiotherapy is currently the mainstay of care for patients with locally advanced OOSCC; however, a growing body of evidence suggests that preoperative chemoradiotherapy may also be a valid alternative for treating OOSCC (Eich *et al*, 2008; Driemel *et al*, 2009). Third, the human papillomavirus (HPV) status was not considered in the subset of patients with oropharyngeal cancer, which may introduce a bias in the estimated effects. However, tumour localization in the oropharynx was not significantly associated with either HCRP1 expression or clinical outcome.

In conclusion, we demonstrated that low HCRP1 expression adversely impacts overall survival and recurrence-free survival in patients with OOSCC. Our findings provide a strong rationale to further evaluate the prognostic significance of HCRP1 expression status in prospective clinical studies in HNSCC.

Conflict of interest

The authors have no financial relationships or conflicts of interest to disclose.

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Authors contribution

Study concept and design: Perisanidis, Filipits, Psyrri, Krainer, Savarese-Brenner, Ewers, Selzer; Literature research: All authors; Experiment: Huynh, Würger, Savarese-Brenner, Wrba; Data collection: Perisanidis, Schopper, Wrba, Würger, Kornek, Selzer; Data control and interpretation: Perisanidis, Filipits, Wrba, Würger; Statistics: Perisanidis, Filipits; Manuscript preparation: Perisanidis, Filipits, Krainer, Savarese-Brenner, Psyrri, Selzer; Manuscript editing and review: All authors.

References

Bache KG, Slagsvold T, Cabezas A *et al* (2004). The Growth-regulatory protein HCRP1/h Vps37A is a subunit of mammalian ESCRT-I and mediates receptor down-regulation. *Mol Biol Cell* **15**: 4337–4346.

Barnes CJ, Ohshiro K, Rayala SK *et al* (2007). Insulin-like growth factor receptor as a therapeutic target in head and neck cancer. *Clin Cancer Res* **13**: 4291–4299.

Braun OM, Neumeister B, Popp W *et al* (1989). Histologic tumor regression grades in squamous cell carcinoma of the head and neck after preoperative radiochemotherapy. *Cancer* **63**: 1097–1100.

Chen LF, Cohen EE, Grandis JR (2010). New strategies in head and neck cancer: understanding resistance to epidermal growth factor receptor inhibitors. *Clin Cancer Res* **16**: 2489–2495.

Ciardiello F, Tortora G (2003). Epidermal growth factor receptor (EGFR) as a target in cancer therapy: understanding the role of receptor expression and other molecular determinants that could influence the response to anti-EGFR drugs. *Eur J Cancer* **39**: 1348–1354.

Cohen EE (2006). Role of epidermal growth factor receptor pathway-targeted therapy in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* **24**: 2659–2665.

Cohen EE, Rosen F, Stadler WM *et al* (2003). Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* **21**: 1980–1987.

Diniz-Freitas M, Garcia-Caballero T, Antunez-Lopez J *et al* (2007). Pharmacodiagnostic evaluation of EGFR expression in oral squamous cell carcinoma. *Oral Dis* **13**: 285–290.

Driemel O, Ettl T, Kolbl O, *et al* (2009). Outcome and histopathologic regression in oral squamous cell carcinoma after preoperative radiochemotherapy. *Strahlenther Onkol* **185**: 296–302.

Egloff AM, Grandis JR (2008). Targeting epidermal growth factor receptor and SRC pathways in head and neck cancer. *Semin Oncol* **35**: 286–297.

Eich HT, Loschcke M, Scheer M *et al* (2008). Neoadjuvant radiochemotherapy and radical resection for advanced squamous cell carcinoma of the oral cavity. *Strahlenther Onkol* **184**: 23–29.

Farshadpour F, Hordijk GJ, Koole R *et al* (2007). Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. *Oral Dis* **13**: 239–243.

Ferlay J, Shin HR, Bray F *et al* (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* **127**: 2893–2917.

Kalyankrishna S, Grandis JR (2006). Epidermal growth factor receptor biology in head and neck cancer. *J Clin Oncol* **24**: 2666–2672.

Karamouzis MV, Grandis JR, Argiris A (2007). Therapies directed against epidermal growth factor receptor in aerodigestive carcinomas. *JAMA* **298**: 70–82.

Karamouzis MV, Konstantinopoulos PA, Papavassiliou AG (2009). Targeting MET as a strategy to overcome crosstalk-related resistance to EGFR inhibitors. *Lancet Oncol* **10**: 709–717.

Katzmann DJ, Odorizzi G, Emr SD (2002). Receptor downregulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol* **3**: 893–905.

Kostelansky MS, Schluter C, Tam YY *et al* (2007). Molecular architecture and functional model of the complete yeast ESCRT-I heterotetramer. *Cell* **129**: 485–498.

Lai MW, Huang SF, Lin SM *et al* (2009). Expression of the HCRP1 mRNA in HCC as an independent predictor of disease-free survival after surgical resection. *Hepatol Res* **39**: 164–176.

Pao W, Miller VA, Politi KA *et al* (2005). Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* **2**: e73.

Perisanidis C, Perisanidis B, Wrba F *et al* (2012). Evaluation of immunohistochemical expression of p53, p21, p27, cyclin D1, and Ki67 in oral and oropharyngeal squamous cell carcinoma. *J Oral Pathol Med* **41**: 40–46.

Pindborg JJ, Reichart PA, Smith CJ, Van der Waal I (1997). Histological typing of cancer and precancer of the oral mucosa. *World Health Organization international histological classification of tumors*. Springer: Berlin.

Rubin Grandis J, Melhem MF, Gooding WE *et al* (1998). Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Inst* **90**: 824–832.

Scully C, Bagan J (2009a). Oral squamous cell carcinoma overview. *Oral Oncol* **45**: 301–308.

Scully C, Bagan J (2009b). Oral squamous cell carcinoma: overview of current understanding of aetiopathogenesis and clinical implications. *Oral Dis* **15**: 388–399.

Silva SD, Cunha IW, Younes RN *et al* (2010). ErbB receptors and fatty acid synthase expression in aggressive head and neck squamous cell carcinomas. *Oral Dis* **16**: 774–780.

Sobin LH, Wittekind C (2002). *International Union Against Cancer (UICC) TNM classification of malignant tumours*, 6th edn. Wiley: New York.

Soulieres D, Senzer NN, Vokes EE *et al* (2004). Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. *J Clin Oncol* **22**: 77–85.

Vermorken JB, Specenier P (2010). Optimal treatment for recurrent/metastatic head and neck cancer. *Ann Oncol* **21**(Suppl 7): vii252–vii261.

Vermorken JB, Mesia R, Rivera F *et al* (2008). Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med* **359**: 1116–1127.

Wheeler DL, Huang S, Kruser TJ *et al* (2008). Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. *Oncogene* **27**: 3944–3956.

Wittinger M, Vanhara P, El-Gazzar A *et al* (2011). hVps37A status affects prognosis and cetuximab sensitivity in ovarian cancer. *Clin Cancer Res* **17**: 7816–7827.